BOTANICAL GAZETTE

FEBRUARY 1908

THE TOXIC ACTION OF CERTAIN ORGANIC PLANT CONSTITUENTS

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(WITH SEVEN FIGURES)

The present paper is the result of a study of the toxic action of certain compounds which, with few exceptions, occur naturally in vegetable tissues. The laboratories of the Bureau of Soils have been engaged for several years in studying the toxic properties of aqueous extracts of unproductive soils. As a result of these studies it has been found, as was shown in *Bulletins 28* and *36*, that many soils are unproductive because of the presence in them of toxic organic compounds. It has also been found that toxic excretions are produced by growing roots (cf. *Bulletin 40*) and that these excretions, if allowed to remain in the soils, may exert a harmful effect upon a second crop of the same or closely related species. Since these excretions are to be regarded, in the light of our present knowledge, as products of plant metabolism which escape from the tissues of the plant, it becomes of some interest to study the action of compounds which are known to exist as such in the tissues of plants and might persist in the soil or arise from related bodies.

Many instructive studies of toxic action of various inorganic and organic compounds upon plants have been made. Those investigators who studied the action of organic compounds have employed, quite naturally, those which are more or less toxic to animal life and to bacteria. As a result, we have much information upon the action of organic acids, phenols, aldehydes, terpenes, cyanogen, and volatile oils. The present study is intended to add some data upon the action

of compounds which occur naturally in plants. A few compounds which have not been found to occur as such in vegetable tissues are included in the study, for reasons which will be evident to the reader.

Material and methods

The organic compounds were dissolved in water and the solutions employed as water cultures for wheat seedlings. Most of the compounds used were obtained from Merck & Co. and were used without further purification. The water used in making up solutions was shaken with washed carbon-black, allowed to stand thirty minutes, and then filtered clear. This treatment, as described by LIVINGSTON (:06), gives physiologically pure water. Continued use of the method proves that it is as efficient in removing the toxic properties of ordinary distilled water as careful redistillation in the presence of strong oxidizing or reducing agents.

The solutions when made were placed in salt mouth glass bottles having a capacity of 250^{cc}, each of which accommodated ten seedling wheat plants. The solutions were made up in the ratio of a certain number of parts per million by weight. Since electrolytic dissociation plays a very subordinate rôle in determining the toxic properties of most organic compounds, the concentration of the solutions was expressed in terms of the mass of the respective substance present. Whenever possible the highest concentration employed was 1000 parts per million, for the sake of brevity designated as "1000 ppm." A number of substances used were soluble only in amounts less than 1000 ppm.; in these cases the range of concentration employed was much smaller, and is noted in the last column of Table II.

The wheat was germinated and water cultures conducted according to the method described by LIVINGSTON (:06) and in *Bulletins 23* and *40* of the Bureau of Soils. The seedlings were put into the water cultures at the stage when the first true leaf was beginning to emerge from its sheath. Twenty plants in two cultures of ten each were used in testing each concentration, and comparison was made with an equal number of plants growing in pure distilled water under like conditions. During the seasons of the year in which the condi-

tions for growth were the best, each experiment was continued for eight to ten days, but during the cloudy winter weather some of the experiments were continued for twelve to fourteen days; the exact length of time for each experiment is given in the third column of Table II.

Two and sometimes three criteria of growth were employed, viz., transpiration, green weight, and turgidity, no one of which can be regarded as absolute; but they usually agree in indicating the order of results, which was the point sought in the experiments. The jars containing the water cultures were closed with cork stoppers notched and carefully fitted to receive the seedlings, so that there was practically no chance for loss of water except that transpired by the seedlings. Therefore, the loss in weight of the cultures represents very closely the amount of water transpired by the plants, and in the case of wheat the transpiration has been shown by LIVINGSTON (:05) and JENSEN (:07) to be a very accurate measure of growth when comparisons are made with control cultures. Transpiration is more nearly proportional to the growth of both roots and tops, while the green weight is not at all an indication of root growth and is liable, moreover, to some error in indicating the actual growth of tops. In computing the effects of the toxic agents employed, the transpiration figures are believed to be a more reliable indication of the growth of wheat, so far as the entire plant is concerned, than the weight of the green tops. Many of the substances employed produced the greatest effect upon the roots of the seedlings, and it was found that the transpiration record was a better indicator of the growth in such cases than the green weight of the tops.

The weight of the tops or of the entire plants in the fresh condition was taken as a second means of measuring their growth and as a means of comparison with the control plants.

The condition of the root tips was also noted as an aid in determining the lethal concentration. When killed, the root tips lose their turgidity and soon become slimy. Before this ensues they generally show indications of injury by discoloration in the region occupied by the most actively growing cells or meristem. In some solutions the tips stop elongating and become swollen, with more or less simultaneous discoloration.

The photographs which are appended show in a general way the growth of the plants in comparison with the controls.

The investigations here reported were intended to reveal the character of the toxic action of the several compounds rather than to establish the exact limits for their toxicity. Strict mathematical accuracy is not claimed for the data, but it is believed that they indicate correctly the order of the results, and that, within the experimental error, they approach the toxic limits for each compound. A general summary of the results is presented in Table II and the details are described in the following pages. This table shows the lowest concentration of the compounds which caused the death of the wheat plants, provided they were killed by a concentration of 1000 ppm. or less; the lowest concentration causing injury; and the concentration causing stimulation, where such a phenomenon was observed.

It would be manifestly improper to make strict comparisons between these results and those obtained by other investigators who determined the concentration necessary to kill or prevent growth in twenty-four hours or less. Obviously, a concentration which is sufficient to check growth in a short interval may cause death in a period of several days.

Experimental results

THE ACTION OF SOME SIMPLE AND SUBSTITUTED AMINO-ACIDS

Recent researches upon the chemistry of proteids have brought the amino-acids into prominence physiologically. These bodies are found in relatively large amounts in plants, especially in those of the legume and mustard families, not only in the seedling stage of the plants but also in the roots, stems, and storage organs of mature plants. According to DOJARENKO (:02) the amino-acid nitrogen forms a very considerable portion of the nitrogen of humus bodies (22 to 70 per cent.).

The action of amino-acids upon plants has been studied by a number of investigators. Asparagin (amino-succinic acid) has received the most attention because of its importance as a nutrient for heterotrophic plants. MAYER ('69) demonstrated the value of asparagin as a nutrient for fungi. LAURENT ('90) studied the effect of glycocoll, leucin, aspartic acid, and asparagin upon yeast. He found that a

1 per cent. solution of glycocoll was harmful to yeast and that 1 per cent. solutions of leucin and aspartic acid were assimilated when carbohydrates were present, but asparagin was assimilated even in the absence of carbohydrate. As classed by LOEW ('99), alanin, leucin, asparagin, and glycocoll form good sources of carbon for bacteria— a classification which is sustained by the result of numerous investigations upon the nutrition of bacteria. NAKAMURA ('96) also showed.

FIG. 1

that asparagin was a favorable nutrient for barley seedlings. KLEBS ('96) found that 1 per cent. solutions of asparagin and glycocoll inhibited zoospore formation in *Conferva minor.*

Our experiments showed that leucin and asparagin are not at all toxic to wheat seedlings. Alanin and glycocoll were slightly injurious at the higher concentrations. Aspartic acid was rather toxic, probably due to its acidic qualities. Tyrosin was more toxic than any of the other amino-acid compounds tried (*fig. 1*). At the expiration of eleven days plants in a solution of tyrosin containing 16 ppm. showed marked injury. At a concentration of 100 ppm. it killed the roots and injured the tops of the plants. At a concentration of 1 ppm. there

was, in some cases, a slight stimulation causing an increase in growth over the controls. The physiological action of different samples of tyrosin was found to vary somewhat.

Upon long standing with exposure to air the tyrosin solutions appeared to have undergone oxidation, all of them having become quite dark in color. The changes wrought were probably the same as those produced by the enzyme tyrosinase, which oxidizes tyrosin to homogentisinic acid, which in turn gives rise to the dark-colored compounds.

An instructive result was obtained by studying the action of such a tyrosin solution which had suffered discoloration incident to age. The tyrosin solution had become so dark in color that it resembled manure extract. It was diluted so that it was equivalent to a 16 ppm. solution of fresh tyrosin. This solution was used for growing wheat seedlings in comparison with a freshly prepared solution of tyrosin of equal strength.

In a few days the plants in the two solutions showed pronounced differences, which became greater as the experiment was continued. *Fig. 1* shows the appearance of the plants when the experiment was discontinued at the end of the twelfth day. The leaves were narrow and beginning to die at the tips; the roots were dead and had become slimy. The plants in the discolored tyrosin had made excellent growth; they resembled plants grown in manure extracts. The leaves were rich green, broad, and making vigorous growth, and the roots showed a similar good development. During the course of the experiment the ten plants in the fresh tyrosin had transpired 19 grams of water, and attained a green weight of 580 milligrams. The plants in the discolored tyrosin had in the same time transpired 38 grams of water and attained a green weight of 980 milligrams.

These results are believed to have importance in explaining the value and action of the so-called green manures used in agricultural practice. The extensive work of E. SCHULZE and others has shown that tyrosin and related compounds are quite widely distributed in the vegetable kingdom. These substances may even increase in number and amount when vegetable matter is allowed to decompose in the soil. Our experiments indicate that bodies like tyrosin and cholin may have a toxic action upon plants, but that these

substances in a more highly oxidized stage are actually beneficial to plant growth.

It is of great interest to note in this connection that green manures may have the same action.   When first applied to the soil they may exert a depressing effect upon the growth of the crop.   After enough time has elapsed for the decomposition of the vegetable matter, with change to other compounds, the crop shows a marked benefit from the action of the green manure.

THE ACTION OF COMPOUNDS RELATED TO LECITHINS

The lecithins are readily decomposed and split off cholin and complex glycerophosphoric acids, which upon further decomposition yield fatty acids, glycerin, and phosphoric acid.   It is such a decomposition that takes place in germinating seeds and also in seedlings in the absence of light.   ZALEWSKI (:o6) believes that this decomposition of the lecithins is due to enzyme action.   He has shown that the organic phosphorus compounds are quickly and almost completely decomposed during germination, only 2 per cent. remaining unattacked. SCHULZE ('91) and other investigators have reported the occurrence of cholin in various plant tissues associated with lecithin, and it does not follow that it must always be regarded as a product of the decomposition of lecithin.

Neurin may arise from lecithin by the life-processes of certain microorganisms, as SCHMIDT and WEISS ('91) have shown.   It may also be formed from cholin by the action of bacteria or by boiling with baryta water.   Betain or oxyneurin may be prepared artificially by the oxidation of cholin.   LOEW ('99) reports that betain is a favorable and cholin is a moderately good source of carbon for bacteria.

In our experiments with wheat seedlings, neurin was found to be very toxic, cholin moderately toxic, while betain was entirely harmless.   Wheat plants were killed by solutions containing 250 ppm. of neurin and injured by solutions containing 25 ppm.   It was thought that at least a part of the injury might be due to the strong basic properties of neurin.   The experiment was accordingly repeated, using neurin which had been neutralized with acetic acid.   The result, however, was not different from the experiment in which the neurin was not neutralized.   It may be possible that a part of the difference in

toxicity may be due to the fact that neurin contains the vinyl group. LOEW ('93) has pointed out that vinylamin ($H_2C = CH \cdot NH_2$) is more toxic to animals than ethylamin ($H_3C \cdot CH_2NH_2$). Substances containing the vinyl group possess the power of forming addition compounds.

Solutions of cholin containing 1000 ppm. did not cause the death of wheat seedlings. Injury was caused, however, by solutions con-
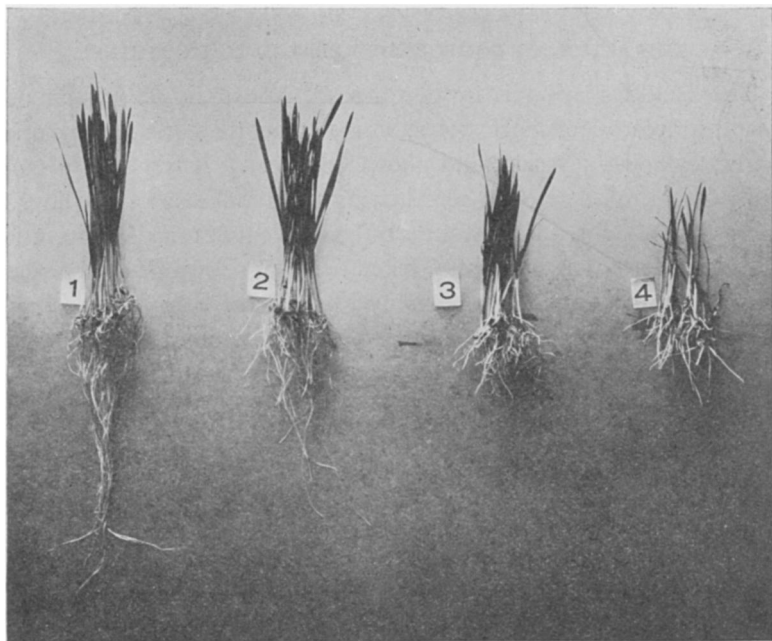
FIG. 2

taining 1000 and 500 ppm. As shown by *fig. 2*, the injurious effect was most marked upon the roots of the wheat plants. The weakest concentration (1 ppm.) caused a slight increase in growth, which was probably due to stimulation.

In contrast to the toxic action of neurin and cholin, the more highly oxygenated betain was not at all toxic to wheat seedlings, but was even slightly beneficial. The concentration of the different solu‑ tions used ranged from 1000 to 5 ppm. and in all of them the plants grew as well or better than the controls in distilled water.

THE ACTION OF SOME UREA DERIVATIVES

Four compounds which are structurally related to urea were studied with regard to their action upon plants. Alloxan and guanidin are sufficiently soluble in water to permit of being used in a concentration of 100 ppm. Guanidin was used as guanidin carbonate. Guanin and xanthin are so slightly soluble in water that the highest concentrations used were only 40 and 25 ppm. respectively.

Guanidin has been shown previously to be toxic to diatoms and filamentous algae by LOEW ('93); toxic to barley seedlings by KAWA-KITA (:04), who also found that some fungi were unable to utilize guanidin as a source of carbon, although they could utilize it as a source of nitrogen. In our experiments with wheat seedlings a concentration of 100 ppm. of guanidin carbonate was sufficient to kill, and all the lower concentrations employed, including 1 ppm., caused serious injury. Alloxan, the ureid of mesoxalic acid, was stated by LOEW ('99) to be an unfavorable source of carbon for bacteria. It is quite toxic to wheat, but less toxic than guanidin. Seedlings were killed by solutions containing 1000 ppm., and injured by lower concentrations down to 100 ppm. Guanin was tested only in concentrations of 40, 20, 10, 5, and 1 ppm. on account of its slight solubility. In all these concentrations there was no harmful effect to be noticed. Xanthin is also so slightly soluble in water that the range of concentrations employed was small. In concentrations of 25, 10, 5, and 1 ppm. it was slightly beneficial to the growth of wheat plants.

THE ACTION OF COMPOUNDS CONTAINING THE PYRIDIN NUCLEUS

Pyridin forms the nucleus upon which are built many of the alkaloids. It is obtained chiefly from coal tar, but may also be obtained from a number of alkaloids (trigonellin, spartein, cinchonin) when these are highly heated, treated with alkalies, or distilled with zinc dust.

It has been observed by FALKENBURG ('91) that the vapor of pyridin and some of its homologues is poisonous to bacteria. The action of pyridin and its homologues has received additional importance as a result of the investigations of SHOREY (:06), who obtained pyridin by the dry distillation of soil. The author cited says (p. 37): "As the fat in this soil was found to be only 0.005 per cent. it does not seem likely that the pyridin formed was due to the formation of

the pyridin ring by condensation, but rather that it exists already in some form in the soil." Pyridin was also obtained from the same soil after extraction with ether, hence it seems that the view taken is the correct one.

In the experiments with wheat seedlings it was found that pyridin, although not sufficiently toxic at a concentration of 1000 ppm. to kill wheat plants in nine days, was nevertheless very injurious, especially to the growth of the green parts of the plants. In a concentration as low as 50 ppm. the growth of the tops was inhibited and the leaf-tips turned brown. In the lower concentrations there was no stimulation of growth.

Picolin ($C_5H_4N \cdot CH_3$), or methyl pyridin, was toxic to wheat plants but acted fatally only in the concentration of 1000 ppm., and caused injury at 500 ppm., but not in lower concentrations. The injury seemed to be manifested by the tops more than by the roots, thus resembling the action of pyridin.

Piperidin ($C_5H_{11}N$), or hexahydropyridin, differs in chemical constitution from pyridin by having two atoms of hydrogen attached to each carbon and one atom of hydrogen attached to the nitrogen atom. Solutions of piperidin killed and injured at a lower concentration than either pyridin or picolin. A sample of piperidin, which was neutralized with acetic acid, proved to be more toxic than the strongly alkaline piperidin itself. Piperidin seemed to injure the roots more severely than the tops. By reference to Table I, it will be seen that piperidin is more toxic than pyridin, a fact which may be due to the additional hydrogen it contains.

Chinolin (C_9H_7N) forms the nucleus of many alkaloids found in plants belonging to the families Rubiaceae and Loganiaceae. FALK-ENBURG ('91) found that chinolin was toxic to bacteria in a concentration of 2000 ppm. Reference to Table II shows that chinolin was sufficiently toxic to kill wheat plants at a concentration of 500 ppm. So low a concentration as 5 ppm. affected them injuriously in six days, but none of the lower concentrations caused stimulation of growth.

PHENOLS

Only a part of the phenols used occur naturally in plants, but in order to make the study more complete several phenols were studied and the results herewith reported.

Monatomic phenols.—Phenol (carbolic acid) is well known on account of its toxic properties, and an enumeration of the literature dealing with its toxicity would be superfluous at this place. Suffice it to say that TRUE and HUNKEL ('98) found that a concentration of $m/400$ phenol prevented the growth of lupine roots. This concentration would be about 235 ppm. and stands quite near the concentration (250 ppm.) which was required to bring about the death of wheat plants subjected to it for eight days.

The wheat plants made a small amount of growth before death in solutions containing 500 and 250 ppm. In solutions containing 100 and 50 ppm. the tops were alive at the end of eight days, but the roots were dead. The plants in solutions of 5 ppm. were slightly poorer than the control plants, but those in 1 ppm. were fully as good as the control plants.

Diatomic phenols.—Pyrocatechin (ortho-dioxybenzene) has been reported in a number of different plants. According to WEEVERS (:03) it occurs in several species of willows and the characteristic black color which the leaves of certain species of willows assume upon drying is probably the result of the oxidation of the pyrocatechin through the activity of an oxidase. YABE ('94) found that pyrocatechin was fatal to yeast cells and bacteria when used in concentrations of 0.4 to 0.58 per cent. TRUE and HUNKEL ('98) found that lupin roots were prevented from growing by a $m/800$ solution of pyrocatechin, i. e., about 137 ppm.

Wheat plants were killed by pyrocatechin in solutions of 500 ppm. and injured by solutions containing as low as 25 ppm. The stronger solutions became dark colored after standing a few days and were undoubtedly somewhat more toxic on that account, as was pointed out by TRUE and HUNKEL ('98). At the conclusion of the experiment the roots of the plants were colored purplish brown by the stronger concentrations. The effects of different concentrations of pyrocatechin are represented in *fig. 3.*

Resorcin (meta-dioxybenzene), which appears to be more stable in solution than pyrocatechin, is less toxic than that compound. TRUE and HUNKEL ('98) found that the growth of lupin roots was prevented by $m/200$ solution (about 550 ppm.), thus exhibiting less toxicity than pyrocatechin. By our method of experimentation we found that the lowest concentration causing death was 1000 ppm. In concentrations

of 500 and 250 ppm. the roots of the plants were entirely dead, although the tops were still alive at the end of the experiment. Concentrations of 100 ppm. stopped the growth of most of the plant roots and adventitious roots from the lower part of the stem ceased to grow as soon as they dipped into the solution. Fifty ppm. was the lowest concentration which appeared to cause injury to the plants.

Hydrochinone (para-dioxybenzene) undergoes oxidation when its solution is exposed for some time to the air. TRUE and HUNKEL ('98)

FIG. 3

found that for lupine roots hydrochinone was more toxic than pyrocatechin or resorcin. With wheat plants the order of results differed somewhat, hydrochinone and pyrocatechin appearing to possess toxicity of about equal intensity. In our experiments a concentration of 500 ppm. of hydrochinone was fatal, 25 ppm. caused injury, and 5 ppm. caused stimulation.

Triatomic phenols.—Phloroglucin (1:3:5 trioxybenzene) is not found as such in plants, but may be derived from several aromatic plant constituents. It was found to suffer some oxidation when plant roots were allowed to grow in its solutions, the stronger solutions show-

ing a distinct yellow color after a few days.   The cultures of wheat seedlings indicated that phloroglucin possesses about the same relative toxicity as pyrocatechin and hydrochinone.   TRUE and HUNKEL ('98) reported that for lupines phloroglucin was somewhat less toxic than either of the two dioxybenzenes mentioned.

In order to study the relative toxicity of phenols containing 1, 2, and 3 hydroxyl groups, a series of cultures was set up in which solutions of phenol, resorcin, and phloroglucin were employed.   It
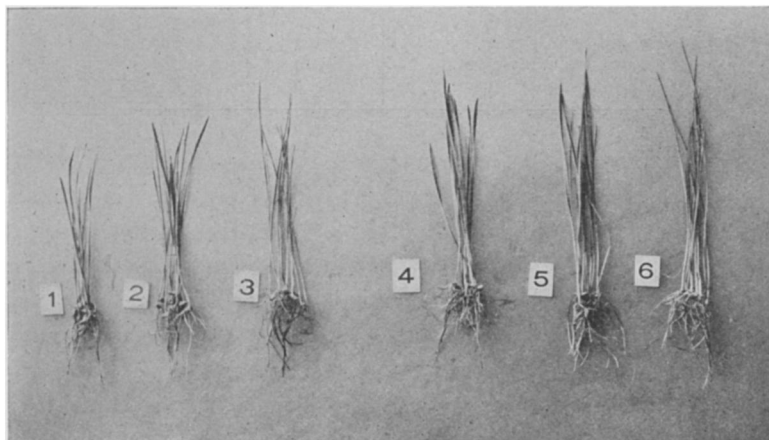

FIG. 4

seemed best to use resorcin and phloroglucin for two reasons.   First, they appear to be more stable in solution; and second, because of the similarity in the arrangement of the hydroxyl groups; resorcin having the 1:3, and phloroglucin the 1:3:5 arrangement.   The cultures were grown under the same conditions for eight days and records of transpiration and green weight taken, although the transpiration is regarded as the more reliable in this experiment.   Table I shows the records for the growth of the plants in the different solutions, and *fig. 4* shows the plants grown in two series of parallel concentration.

It would appear from these figures that the toxicity of phenols is not directly dependent upon the number of hydroxyl groups they contain.   Resorcin appears to be the least toxic of the three compounds and phenol the most toxic.   TRUE and HUNKEL found that

TABLE I

Growth of wheat plants in solutions of phenol, resorcin, and phloroglucin

Solution	Transpiration grams	Green weight grams
Phenol 500 ppm............	dead	....
Phenol 250 ppm............	dead	....
Phenol 100 ppm............	20.6	0.93
Phenol 50 ppm............	27.8	0.64
Resorcin 500 ppm...........	19.9	0.53
Resorcin 250 ppm...........	23.8	0.76
Resorcin 100 ppm...........	26.4	0.84
Resorcin 50 ppm...........	41.0	0.98
Phloroglucin 500 ppm.......	15.0	0.54
Phloroglucin 250 ppm.......	16.7	0.69
Phloroglucin 100 ppm.......	22.9	0.98
Phloroglucin 50 ppm.......	31.0	0.80

the toxic limit of phenol was not altered by the addition of sodium hydroxid, hence the greater toxicity of this compound is probably not due to its acid properties, but is to be attributed to the properties of the phenyl group. The introduction of additional hydroxyl groups into phenol appears to alter its toxicity variously, so far as it is possible to judge of the toxicity of the different unstable compounds. Pyrogallol was not studied in this connection, because it becomes converted upon standing to purpurogallein, which is more toxic, according to TRUE and HUNKEL, than pyrogallol.

There seems to be no evidence, therefore, from our experiments that the toxicity of phenols to wheat is diminished as the number of hydroxyl groups is increased.

DERIVATIVES OF SOME OXYBENZENES

Arbutin, which may be regarded as a glucoside of hydrochinone, is quite widely distributed among plants, especially among members of the Ericaceae.

LAURENT ('90) found that arbutin seemed to be assimilated in small quantities by yeast. KLEBS ('96) found that a 0.5 per cent. solution inhibited zoospore formation in *Conferva minor*, although it did not appear directly to cause the death of alga cells in the first day.

In studying the action of arbutin upon wheat seedlings, concentrations of 1000, 500, 100, 25, and 1 ppm. were employed. At the end of three days it was noticed that the wheat roots growing in the

stronger solutions had oxidized part of the arbutin to an insoluble, light-yellow dye which was deposited upon the roots.   When the experiment was discontinued at the end of twelve days, the plants in arbutin solutions containing 1000 and 500 ppm. were dead.   The roots of these plants were discolored at the tips and had become slimy. The plants in solutions containing 100 and 25 ppm. were alive, but had made much less growth than the controls in distilled water. The plants in the solutions containing 1 ppm. of arbutin had been stimulated to make slightly better growth than the control plants.
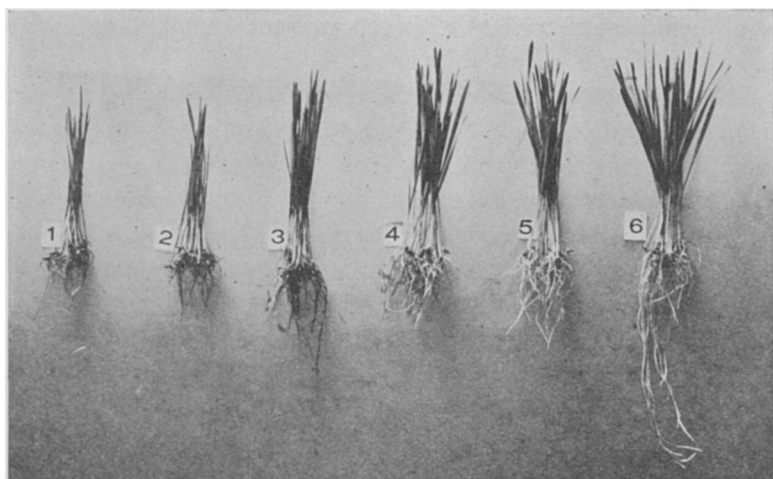

FIG. 5

Vanillin, the aromatic principle of the vanilla bean, is a methoxy-ether of protocatechuic aldehyde.   It probably does not occur as such in plants, but exists in the form of a glucoside which breaks into vanillin and a sugar when the plant organs are dried.

KLEBS ('96) found that saturated and half-saturated solutions of vanillin were fatal to *Conferva minor*.   Our experiments showed that wheat seedlings were killed in nine days by solutions containing 1000 and 500 ppm. of vanillin.   In solutions containing 100 ppm. of vanillin the tops made fairly good growth, but the roots were killed. In the most dilute solutions, i. e., 10 ppm. and less, there was sometimes a slight stimulation of growth (*fig. 5*).

The roots of the plants grown in the stronger solutions oxidized some of the vanillin to a purplish-brown, insoluble dye, which colored the roots. The general character of the oxidation has been described in a preliminary communication by SCHREINER and REED (:07c).

Vanillic acid, a monomethylprotocatechuic acid, showed greater toxicity to wheat plants than vanillin. The plants were killed in seven days in solutions of 100 ppm. or stronger. In solutions containing 25 ppm. of vanillic acid, the only inferiority noticed in the plants was their root development; but the plants grown in concentrations of 5 and 1 ppm. were better than the controls in distilled water. Although vanillic acid is more toxic to wheat plants than vanillin, it appears to be less inhibitory of root development.

Chinone (benzochinone) may be prepared artificially from quinic acid or from hydrochinone. It is a compound of interest in the study of soil problems since BEIJERINCK (:00) has found that a soil fungus, *Streptothrix chromogena,* has the ability to form chinone from proteids.

Chinone has been shown to be toxic to the seedlings of various plants, algae, and fungi by FURUTA (:02). In concentrations of 1000 and 500 ppm. the roots of soy bean and wheat seedlings were injured within a few hours and killed in three or four days. Filaments of algae were killed in a concentration of 1000 ppm. in three or four hours, and fungi in a somewhat longer period.

Chinone is one of the most toxic compounds whose effect upon wheat seedlings was studied. A concentration of 100 ppm. was fatal in nine days, and even 1 ppm. produced an injurious effect. In the intermediate concentrations the chinone was correspondingly injurious. The plants scarcely survived in a concentration of 50 and 25 ppm. and only in concentrations of 10 and 1 ppm. was there any growth comparable to that of the controls in distilled water.

The great toxicity of chinone is probably due to two of its chemical properties. The first of these is its strong oxidizing power, by virtue of which it is probably able to oxidize labile compounds which exist in cells of the plants and render them unsuitable for use in metabolism. The second property which gives chinone a toxic action is its ketone nature. Chinone readily forms bromin addition products. It unites with one molecule of hydroxylamin to form chinonoxime; with two molecules of hydroxylamin it forms chinondioxime.

The ketones, as is well known, are distinctly toxic to plants, and taken together these two properties undoubtedly account for the action of chinone upon plants.

Cinnamic acid occurs in small amounts in the secretions of certain plants and its esters are found in the leaves of various tropical plants. By proper treatment it yields all the different phenol acids.

TRUE (:00) found that a concentration of cinnamic acid equivalent to $m/12,800$ (12 ppm.) prevented the growth of the root tips of *Lupinus albus* in 24 hours. He also found that sodium cinnamate was toxic to root tips when employed in a concentration of $m/800$ (184 ppm.).

Using a slightly different method, the results obtained with wheat seedlings varied, naturally, from those obtained with lupine. The same relations, however, hold true in both cases. Cinnamic acid is strongly toxic for seedlings, and its sodium salt is moderately toxic.

A concentration of 100 ppm. of cinnamic acid was fatal to wheat plants and 25 ppm. was injurious. Cinnamic acid is particularly toxic to root growth, and it was only in the concentrations below 25 ppm. that anything like normal root growth was exhibited. When a wheat root is killed by cinnamic acid it is not swollen nor discolored in the usual fashion, but on the contrary appears perfectly white.

Sodium cinnamate is much less toxic than cinnamic acid. Wheat plants were not killed by a concentration of 1000 ppm. and were not injured at concentrations weaker than 100 ppm.

Cumarin is found in a number of different plants and gives the odor which is characteristic of Hierochloa and Melilotus. It is a lactone of cumaric acid which may be regarded as orthoxycinnamic acid. It is also of interest to note that GOSIO (:06) has found that cumarin may be formed from carbohydrates by the action of certain mold fungi, e. g., *Aspergillus glaucus, A. novus, A. flavescens,* and *A. varians.*

Orthocumaric acid and *o*-hydrocumaric acid (melilotic acid) are found in Melilotus (sweet clover) in addition to cumarin. Para-hydrocumaric acid, although it has not been reported in plants, is of interest on account of its relation to tyrosin. Through the action of bacteria, the amid group in tyrosin is broken up and ammonia liberated, thus giving rise to *p*-hydrocumaric acid.

KLEBS ('96) found that *Conferva minor* was quickly killed in

saturated and half-saturated solutions of cumarin. Our results show that cumarin is extremely poisonous to wheat plants. At the end of five days the plants in 250 ppm. and stronger were dying, the roots and tops having made practically no growth. The root tips were swollen and slightly discolored, although the roots themselves were quite turgid. When the experiment was discontinued at the end of eight days the plants in the solutions of 100 ppm. were dead, although

FIG. 6

they had made a slight growth at the beginning of the experiment. The roots were discolored for a distance of 3 to 6^{mm} from the tip and their surface was very slimy, due to the death of the outer layers of cells which were then beginning to peel off. The leaves of the affected plants were short and broad, a feature not brought out by the illustrations, but which was very characteristic in all experiments with cumarin. The meristematic tissue of the stem appeared to be injured in such a way that only the first leaves were unfolded, and in most cases the sheathing leaf base was more or less swollen by the abnormal growth of the inhibited leaves within it.

The plants in the solutions of 50 ppm. were alive at the end of

eight days, but all growth had ceased.   The root tips were discolored and badly swollen.   The leaves were dead for a considerable distance back from the tips.   In the solution of 1 ppm. the development of tops, as shown by the illustration (*fig. 6*), was practically equal to that of the control plants in distilled water.   The root development was not as good, however, the tips being slightly discolored but not swollen.

Daphnetin, which occurs in various species of Daphne, is a lactone, which is to be regarded as (3, 4) dioxycumarin.   On account of its slight solubility in water it was impossible to test daphnetin in concentrations greater than 50 ppm.   Daphnetin was relatively less toxic than cumarin.   At a concentration of 50 ppm. the green weight of tops produced in twelve days was 76 per cent. of the controls. In concentrations of 25 ppm. and less the growth of tops was practically as good as in distilled water, and the development of roots was only slightly inferior.

Esculin is of particular interest on account of its relation to cumarin. It is the glucoside of esculetin, which is a lactone isomeric with daphnetin, and has been shown by TIEMANN and WILL ('82) to be (4, 5) dioxycumarin.

Both esculin and esculetin have been found in plants, but little is known concerning their distribution.   The esculin solutions had a blue fluorescence when prepared, which was lost after plants had grown for a time in them.   The roots which grew in the stronger solutions were colored dark yellow as a result of their oxidative activities, the dye formed being insoluble and remaining upon the surfaces of the roots where oxidation had occurred.   This effect is shown in *fig. 7*.   Esculin, as shown by the figures, is decidedly less toxic to plants than cumarin, but more toxic than daphnetin.   KLEBS ('96) found that a saturated solution of esculin inhibited the formation of zoospores in *Conferva minor*, but did not prevent the formation of chlamydospores in *Mucor racemosus*.   The toxic effects of esculin were more marked upon the roots of wheat plants than upon the tops. In concentrations of 1000 and 500 ppm. wheat plants were killed, and were injured even in a solution containing 1 ppm. (*fig. 7*).   In the solutions containing 100 ppm. there was a distinct stimulation of the growth of tops.

Heliotropin, or piperonal, is the aldehyde of piperonylic acid. It possesses the pleasant odor of heliotrope. The growth of wheat plants in solutions was markedly affected by the presence of heliotropin. Although the plants were not killed by a concentration of 1000 ppm. or less, they were injured by such slight quantities as 1 ppm. It is worthy of remark that the tops of the wheat plants were more affected by the heliotropin than the roots. The roots were healthy and of nearly equal development in all solutions of heliotropin used.
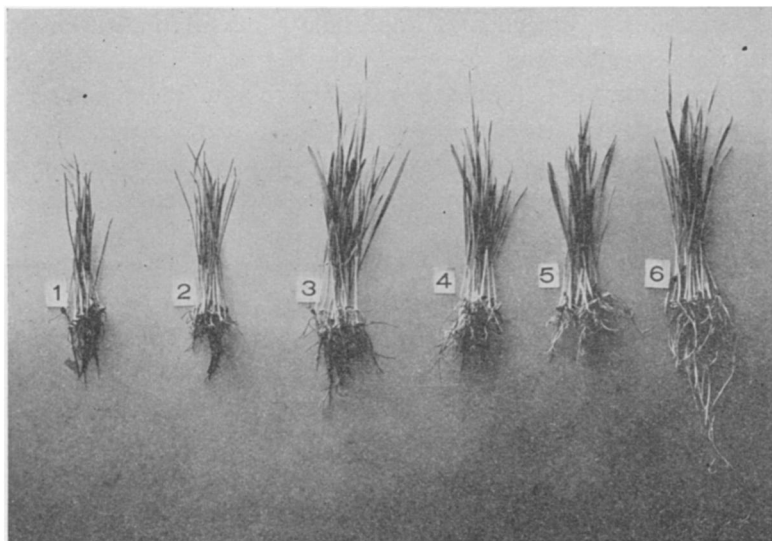

FIG. 7

MISCELLANEOUS COMPOUNDS

A few of the compounds whose effects were studied do not permit of classification with the majority of compounds used. These bodies are skatol, ricin, mucin, and quinic acid.

In solutions skatol proved to be somewhat toxic to wheat plants. A concentration of 200 ppm. was sufficient to kill seedling plants in nine days, and in the same time a concentration of 50 ppm. was injurious. In those cases where skatol had a harmful effect, the roots of the wheat plants were more injured than the tops.

Ricin is the very poisonous (for animal life) principle of Ricinus;

its chemical composition is unknown, but it is probably a toxalbumen. BOKORNY ('96) found that ricin is slightly toxic to algae and infusoria. On account of its slight solubility, we were not able to make tests of solutions having a concentration greater than 50 ppm.   The wheat plants were somewhat injured by concentrations of 50 and 40 ppm.

Mucin is also comparatively insoluble in water and hence a wide range of concentrations could not be employed. In a saturated solution there was practically no growth of the roots of the wheat plants. In a solution of 100 ppm., which is slightly below the point of saturation, the plants were seriously injured in the growth of both roots and tops.   A solution containing 5 ppm. was slightly stimulating to the growth of wheat plants.

Quinic acid, which occurs in cinchona bark associated with the alkaloid quinin, proved to be quite toxic to seedlings.   In all except the more dilute solutions, quinic acid was very toxic to wheat plants. At the end of ten days the entire plants in concentrations of 1000 and 500 ppm. were dead; in 100 ppm. only the roots were dead.   In concentrations below 50 ppm. the plants were as good as the controls grown in distilled water; and in the solutions containing 5 ppm. there was a slight stimulation of growth.

Discussion and summary

The data presented show that a number of the compounds occurring naturally in plants have toxic effects upon wheat seedlings.   The toxicity of certain compounds appears to be greater for plants than for animals; in the case of other compounds the reverse relations seem to exist.
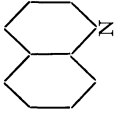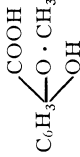
The amino-acid compounds display a relatively low toxicity, and some of them appear to serve directly as nutrients to the plants. Two exceptions deserve mention. Tyrosin, which was found to cause injury in concentrations of 25 to 10 ppm., may be regarded as oxyphenylalanin.   Reference to the table shows that alanin itself was slightly injurious in the highest concentrations, and it is easy to understand that increased toxicity should follow the introduction of the oxyphenyl group.   Aspartic acid is the other amino-compound which displays relatively great toxicity to the plants.   Aspartic acid differs chemically from the other amino-compounds employed in

BOTANICAL GAZETTE

TABLE II

Effect of various organic compounds upon the growth of wheat plants (ppm = parts per million).

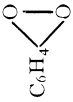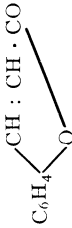| No.	Compound	Duration of experiment in days	Lowest concentration causing death	Lowest concentration causing injury	Concentration causing greatest stimulation	Remarks
1	Glycocoll $CH_2(NH_2)COOH$	9	......	......	......	Tops of all plants good. Roots sl'tly inj'd at higher conc'tions.
2	Alanin $CH_3 \cdot CH(NH_2) \cdot COOH$	10	......	500 ppm	25 ppm	Only the roots were injured by concentration of 500 ppm.
3	Leucin $CH_3 \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	9	......	......	......	No injurious action.
4	Tyrosin $C_6H_4 \Big\langle {\scriptstyle OH \atop CH_2 \cdot CH(NH_2) \cdot COOH}$	11	......	10 ppm	......	
5	Aspartic acid $HOOC \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	10	500 ppm	100 ppm	......	Normal growth in concentrations below 100 ppm.
6	Asparagin $NH_2OC \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	9	......	......	......	No injury in concentrations below 1000 ppm.
7	Neurin $(CH_3)_3N \Big\langle {\scriptstyle CH:CH_2 \atop OH}$	9	250 ppm	25 ppm	......	
8	Neurin (neutralized)	8	250 ppm	25 ppm	......	
9	Cholin $(CH_3)_3N \Big\langle {\scriptstyle CH_2 \cdot CH_2OH \atop OH}$	10	......	500 ppm	1 ppm	Roots were injured more than the tops.

No.	Name / Structure					Remarks
10	Betain $(CH_3)_3N\!<\!{}^{CH_2\cdot CO}_{O}$	9	......	......	......	No injury.
11	Alloxan $CO\!<\!{}^{NHCO}_{NHCO}\!>\!CO$	10	1000 ppm	100 ppm	......	
12	Guanin $NH_2\cdot C\cdot NH\cdot CO\cdot C\cdot NH$; $N\!=\!C\!-\!N\!=\!CH$	12	......	......	......	Insoluble above 40 ppm. No harmful effects at that concentration.
13	Xanthin $CO\cdot NH\cdot CO\cdot C\cdot NH$; $NH\!-\!C\!-\!N\!=\!CH$	10	......	......	......	Insoluble above 25 ppm. No injurious effects at that concentration.
14	Guanidin $HN:C\!<\!{}^{NH_2}_{NH_2}$	9	100 ppm	1 ppm	......	
15	Pyridin C_5H_5N	9	......	50 ppm	......	In solutions of 50 ppm and less, the root growth was normal.
16	Picolin $C_5H_4N\cdot CH_3$	7	1000 ppm	500 ppm	100 ppm	
17	Piperidin C_5H_4N (ring: CH_2, H_2C CH_2, H_2C CH_2, NH)	7	250 ppm	25 ppm	......	

TABLE II—*Continued*

No.	Compound	Duration of experiment in days	Lowest concentration causing death	Lowest concentration causing injury	Concentration causing greatest stimulation	Remarks
18	Piperidin (neutralized)	7	100 ppm	25 ppm	1 ppm	
19	Chinolin	6	500 ppm	5 ppm	......	
20	Phenol $C_6H_5 \cdot OH$	11	250 ppm	5 ppm	......	
21	Pyrocatechin $C_6H_4(OH)_2$, 1 : 2	12	500 ppm	25 ppm	1 ppm	
22	Resorcin $C_6H_4(OH)_2$, 1 : 3	10	1000 ppm	50 ppm	1 ppm	
23	Hydrochinone $C_6H_4(OH)_2$, 1 : 4	9	500 ppm	25 ppm	5 ppm	
24	Phloroglucin $C_6H_3(OH)_3$, 1 : 3 : 5	13	500 ppm	25 ppm	1 ppm	
25	Arbutin $C_{12}H_{16}O_7$	12	500 ppm	25 ppm	1 ppm	
26	Vanillin $C_6H_3\!<^{CHO}_{O \cdot CH_3}\!>OH$	9	500 ppm	1 ppm	......	
27	Vanillic acid $C_6H_3\!<^{COOH}_{O \cdot CH_3}\!>OH$	7	100 ppm	25 ppm	5 ppm	

No.	Name and formula				Remarks
28	Chinone C_6H_4<$\,^O_O$>	9	100 ppm	1 ppm	. . . . . .	
29	Cinnamic acid $C_6H_5 \cdot CH : CH \cdot COOH$	8	100 ppm	25 ppm	. . . . . .	Roots were stimulated in lower concentrations.
30	Sodium cinnamate $C_6H_5 \cdot CH : CH \cdot COONa$	12	. . . . . .	100 ppm	. . . . . .	
31	Cumarin C_6H_4<$\,^{CH:CH \cdot CO}_O$>	8	100 ppm	1 ppm	. . . . . .	
32	Daphnetin C_6H_2<$\,^{CH:CH \cdot CO}_O$>$(OH)_2$	12	. . . . . .	50 ppm	. . . . . .	Insoluble above 50 ppm. Roots injured more than tops by 50 ppm.
33	Esculin $C_{15}H_{16}O_9$	13	500 ppm	1 ppm	. . . . . .	
34	Piperonal (heliotropin) C_6H_3<$\,^O_O$>CH_2 CHO	7	100 ppm	1 ppm	. . . . . .	
35	Skatol C_6H_4<$\,^{C \cdot CH_3}_{NH \cdot CH}$>	9	200 ppm	50 ppm	. . . . . .	Roots injured more than tops.
36	Ricin	10	. . . . . .	40 ppm	. . . . . .	Insoluble above 50 ppm.
37	Mucin	10	. . . . . .	100 ppm	. . . . . .	Not tested in solutions stronger than 100 ppm on account of insolubility.
38	Quinic acid $C_6H_7(OH)_4 \cdot COOH$	10	500 ppm	100 ppm	. . . . . .	

containing two carboxyl groups, only one of which is linked with the amid group. The behavior of the seedlings grown in solutions of aspartic acid is in harmony with this fact. The injurious effect of the aspartic acid suddenly disappears as soon as diluted below 100 ppm. and the gradual disappearance of toxic effects is not shown. The toxicity of aspartic acid, therefore, seems to be due largely to the carboxyl groups which it contains.

The physiological action of neurin, cholin, and betain presents some interesting facts bearing upon the relation of toxicity to chemical structure. It will be noted, by reference to the structural formulae for these compounds, that they differ only in one of the groups. Neurin contains the ethylene group; cholin contains an ethyl in place of the ethylene group, and has one carbon atom oxidized to the primary alcohol stage; finally in betain the alcohol group is oxidized to carboxyl with subsequent splitting-off of water. Corresponding to the increased degree of oxidation in these three compounds, there is a decrease in their toxicity, neurin being the most toxic, and betain being absolutely non-toxic in concentrations less than 1000 ppm.

On account of the slight solubility of two of the four urea derivatives employed, it is not possible to make comparisons of their physiological actions. The compounds which contain the purin group did not appear to be toxic. Guanidin, however, is distinctly toxic, and alloxan moderately toxic.

The pyridin compounds employed are all toxic, some of them being fatal to wheat plants in relatively small amounts. Pyridin itself appears to be relatively less toxic than any of the other related compounds studied. It was not fatal at a concentration of 1000 ppm. and only injured the tops slightly in concentrations of 100 ppm. and less. The introduction of methyl into the pyridin appears to increase slightly its toxicity (picolin). The addition of more hydrogen to pyridin (reduction) produces a marked increase in its toxicity, as is shown by the physiological action of piperidin, which may be regarded as hexahydropyridin. This fact supports what was said on a previous page concerning the decrease of toxicity accompanying the oxidation of neurin, cholin, and betain. Chinolin appears to be somewhat more toxic than pyridin, as might be expected from its resemblance to naphthalene.

The phenol compounds studied displayed varying degrees of toxicity. In general their toxicity does not appear to depend upon the number of hydroxyl groups they contain. Phenol itself is the most toxic of any of the members of that group which have been reported in this paper. Undue credence, however, must not be given to slight differences in the toxicity of the di- and triatomic phenols, because it is known that they are more or less unstable in solution, and as a result may be converted into more toxic compounds.

The toxicity of cinnamic acid is not difficult to understand when one notes that it is phenylacrylic acid. Sodium cinnamate is much less toxic than the corresponding acid, indicating at least that a part, but not all, of the injurious properties of cinnamic acid are due to the amount of carboxyl it contains. TRUE (:oo) reported that of a series of sodium salts of organic acids, sodium cinnamate was the most harmful to lupine seedlings. This observation indicates still more strongly that the phenylacrylic group is responsible for a considerable part of the toxicity exhibited by cinnamates.

Cumarin is related to cinnamic acid, and it is worthy of note that this lactone, containing no dissociable hydrogen, is as toxic as cinnamic acid containing dissociable hydrogen.

Daphnetin is another compound which shows a diminished toxicity accompanying a higher stage of oxidation. It differs from cumarin in having two atoms of phenyl-hydrogen replaced by hydroxyl, with the result that the toxicity is somewhat diminished.

While it would be presumptuous to assert that decrease in toxic action always accompanies the introduction of oxygen into organic compounds, it is undoubtedly true that in a number of the cases studied, the more highly oxygenated compounds are less toxic than those containing less oxygen. Many of these substances which are ordinarily regarded as by-products in vegetable metabolism are highly toxic to seedlings when present in sufficient quantities.

It will be noted that certain of the compounds studied produced the effect which LE RENARD (:02) has termed "chemauxesis" or stimulation, a phenomenon which has been studied carefully by RAULIN ('69), RICHARDS ('97, '99), ONO (:oo), and others. RICH-ARDS found in the case of the fungi he studied that the presence of a

small amount of toxic agent enabled the plants to work more economi-
cally with a given amount of nutritive material. It is possible, when
toxic agents are present in small amounts, that they excite the cells
to greater activity, as a result of which the cells are able to utilize
more fully the materials at their disposal, or to overcome retard-
ing conditions of their environment. Such an action would be
analogous to the "activating" influence of certain inorganic salts upon
enzymes.

The stimulating agents may also act beneficially upon growth
by virtue of the changes they cause in the excretory matter of the
plant or of the cell. The writers have shown (:07a, :07b) that
deleterious waste products are excreted from living plants, and that
the accumulation of these substances may be detrimental to vegetable
growth unless some agency removes or destroys the excretory prod-
ucts, either by the formation of harmless compounds, or more simply
by precipitating them. It is well known that salts of the heavy
metals, which in small amounts exert a stimulating action on plants,
will also bring about a precipitation of proteid-like bodies. When
larger amounts are present, these substances not only combine with
the excreted proteid bodies at the surface of the plant, but also
exert this same action on the living protoplasm within the plant,
causing death.

If the excretory products mentioned be of the nature of proteids,
it is easy to see how beneficial conditions might arise by the continual
precipitation of the harmful excretions. In other cases it is possible
that the compounds which exert a stimulating action may set up
chemical processes which, while not precipitating excretions, never-
theless render them harmless to the plants which produce them.

From this point of view, the stimulation of plant growth by small
quantities of toxic agents consists in the removal of deleterious waste
products, and the maintenance of a sanitary environment for the
growing plant. It is not at all improbable that each of the processes
mentioned may act as factors in increasing plant growth at different
times and under different conditions.

The oxidizing power of the plant roots was shown in several
instances by the formation of colored compounds which stained the
roots.

Certain of the substances employed appear to have had a toxic action mainly upon the growth of the tops of the plants; other substances were more toxic to the growth of roots than of the tops.

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